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Tertiary Hydrogen Bonds in the Solution Structure of Transfer RNA

(base pairing/high resolution nuclear magnetic resonance)

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ABSTRACT **The high resolution nuclear magnetic resonance (NMR) spectra of hydrogen-bonded protons in four tRNAs have been studied at 270 MHz. The relative intensity of the resonances between −11 ppm and −15 ppm of *Escherichia coli* $tRNA_1^{Val}$ indicate that there are 26 ± 3 protons, while only 20 are expected from secondary structure Watson–Crick hydrogen bonds in the cloverleaf structure. Several possible candidates for these extra resonances are suggested by tertiary interactions observed in recent crystallographic studies.**

Of the four tRNAs studied, three, e.g., *E. coli* $tRNA_1^{Val}$, *E. coli* $tRNA^{Arg}$ and *E. coli* $tRNA^{Phe}$ have one "GU pair" in their cloverleaf structure, while the fourth, yeast $tRNA^{Asp}$, has three "GU pairs" and one "GΨ pair". Correlating these with the NMR spectra in the −10 ppm to −11 ppm region allows us to conclude that the "GU pairs" are not hydrogen-bonded by tautomerization to the lactim form.

At the very low field region, near −14.9 ppm, the three *E. coli* tRNAs show a single resonance which is attributed to the 4-thiouracil 8 to adenine 14 hydrogen bond of the tertiary structure, by analogy with the recent crystal structure of yeast $tRNA^{Phe}$. This assignment is confirmed by the disappearance of this resonance after treatment with cyanogen bromide.

Previous studies indicate that the low field (−11 to −15 ppm) nuclear magnetic resonance (NMR) spectra of nucleic acids contain contributions from one proton in the ring NH hydrogen bond of each base pair (1–3) of the secondary structure. Recent studies on *Escherichia coli* $tRNA^{Glu}$ assigned one additional resonance to a tertiary structure hydrogen bond (4); however, the numerous hydrogen bonds from tertiary interaction seen in the recent x-ray crystal structures (5, 6) have not previously been apparent in the NMR spectra of the tRNAs. It is obvious that NMR spectra of tRNAs with greater resolution would allow a more reliable assignment of the resonances, including resonances in the low field region from other kinds of exchangeable protons, as well as possible additional resonances from the tertiary structure. Assignment of the resonances in the yeast $tRNA^{Phe}$ spectrum (3), together with the spectra of other tRNA species, led to the generation of a set of ring current shift rules which approximately predict the observed resonance positions (7). These assignments have been strengthened by recent NMR measurements at various temperatures where sequential melting of several different tRNAs was observed (4, 8). The resulting first order understanding of the low field resonance positions allowed us to identify tRNA species of known sequence which were predicted to give inherently better resolved spectra; such well-resolved spectra would hopefully reveal additional tertiary resonances if they were present. In the present paper we take advantage of the better spectral resolution obtained by the use of highly purified tRNA samples, some which have not been studied before, and improved magnetic field homogeneity at 270 MHz, to examine, and in some cases assign, previously unassigned resonances between −9 and −10 ppm, between −10 and −11 ppm, and between −11 and −15 ppm. For this purpose we have used purified samples of *E. coli* $tRNA_1^{Val}$, *E. coli* $tRNA^{Arg}$, *E. coli* $tRNA^{Phe}$, and yeast $tRNA^{Asp}$. The tertiary structure of yeast $tRNA^{Phe}$ determined crystallographically has been used as a possible model for the additional resonances observed in these tRNAs.

Abbreviations: NMR, nuclear magnetic resonance.

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MATERIALS AND METHODS

$tRNA_1^{Val}$. *E. coli* $tRNA_1^{Val}$ was purified by BD-cellulose chromatography (9), DEAE-Sephadex chromatography (10), and RPC-5 (reverse phase) chromatography (11). The final material was 98% pure according to the stoichiometry of aminoacylation with L-[^{14}C]valine by partially purified *E. coli* valyl-tRNA synthetase. The starting material in the experiments in which the sulfur was removed from $tRNA_1^{Val}$ was a different preparation with a purity of 89%. *E. coli* $tRNA^{Phe}$ and *E. coli* $tRNA^{Arg}$ were purified by the same procedures to an aminoacylation stoichiometry of 95% and 99%, respectively; the latter, although pure with respect to arginine acceptor activity, is a mixture of more than one iso-accepting species. Yeast $tRNA^{Asp}$ was a gift of Dr. G. Dirheimer.

NMR Spectra. Samples (5 mg) of tRNA were dissolved in 0.19 ml of buffer to give a concentration of about 1.1 mM. Spectra were obtained using micro NMR tubes 5 mm diameter, 8 mm high; the solvent was 10 mM Na cacodylate, pH 7.0, 1 mM EDTA, 0.1 M NaCl, 15 mM $MgCl_2$, i.e., the Mg^{2+}:tRNA ratio was about 12. A Bruker 270 MHz spectrometer was used in the continuous wave mode to obtain spectra which were time-averaged for 60 to 100 sweeps of 50 sec each.

Removal of Sulfur from tRNA. The sulfur was removed from the unique 4-thiouridylyl (s^4U) residue at position 8 in $tRNA_1^{Val}$ by reaction with cyanogen bromide at pH 8.5 under the conditions described by Walker and RajBhandary (12). The tRNA solution was adjusted to an A_{260} of 20 in the incubation buffer (about 32 μM tRNA); addition of cyanogen bromide to a concentration of 50 μM was usually sufficient to convert at least 80% of the s^4U to U during the 4 min incubation. After incubation the tRNA was cooled to 0°, dialyzed twice against 4 liters of water, and lyophilized prior to determining ultraviolet spectra and amino-acid acceptor activity.

Aminoacylation Assays. The incubation medium contained (in a volume of 0.2 ml); 20 μmol of Tris·HCl pH 7.5, 10 μmol of $MgCl_2$, 2 μmol of ATP, 2 μmol of reduced glutathione, 40 μg

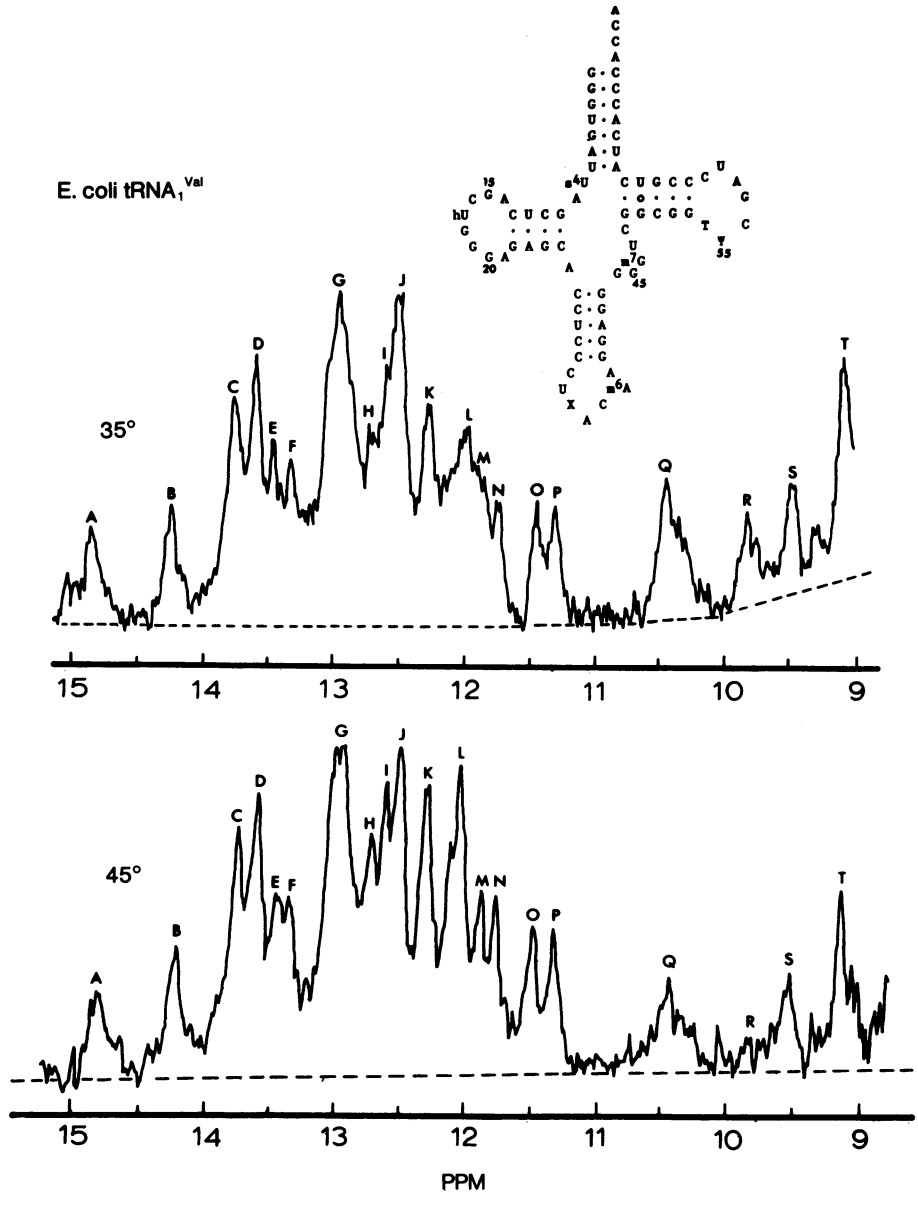

FIG. 1. 270 MHz proton NMR spectrum of *E. coli* tRNA$_1^{Val}$ at 35° and 45°.

of bovine serum albumin, 0.016 μmol of L-[^{14}C]valine (specific activity 9000 cpm/μmol), 5 units of partially purified valyl-tRNA synthetase (1 enzyme unit catalyzes the incorporation of 1 nmol of valine into tRNA in 10 min at 37°) and about 0.5 A_{260} units (about 0.8 nmol) of tRNA. The mixture was incubated at 37° and 40 μl aliquots were removed at 1, 4, 7, and 10 min and pipetted onto filter paper discs which were dropped immediately into ice-cold 10% trichloroacetic acid and washed, dried, and analyzed for radioactivity as described previously (13). The maximum plateau value of incorporated radioactivity was transformed to nmol of valine and converted to nmol incorporated per nmol of tRNA.

RESULTS

Resonances between −9 and −10 ppm

Fig. 1 shows the low field NMR spectrum of *E. coli* tRNA$_1^{Val}$ at 35° and at 45°. The spectrum is very well resolved, showing 20 individual peaks between −9 ppm and −15 ppm. The spectral region between −9 ppm and −10 ppm, i.e., closer to the large H_2O resonance, is well resolved and, at 35°, contains three new peaks at −9.8 ppm, −9.5 ppm, and −9.1 ppm with intensities in the approximate ratio of 1:1:2. Although resonances had been observed in this region previously (2) the present spectrum with its better baseline and narrower peaks allows more accurate relative intensity measurements. Similar peaks are also seen in three other tRNAs in Fig. 2, but the resolution was better for the *E. coli* tRNA$_1^{Val}$ sample. Possible origins of these resonances are discussed below.

Resonances between −10 and −11 ppm

In Fig. 2 the region between −10 and −11 ppm has a very flat baseline so that it can be seen that the three tRNAs from *E. coli* each have a single resonance in this region. Integration with respect to resolved single resonances of tRNA^{Val}, or with respect to the large area between −11 and −15 ppm

shows that the intensities of these peaks correspond to two protons. The bottom spectrum in Fig. 2, from yeast $tRNA^{Asp}$, has an intensity in this region corresponding to about 10 protons. *E. coli* $tRNA_1^{Val}$ (14, 15), *E. coli* $tRNA^{Phe}$ (16), and *E. coli* $tRNA^{Arg}$ (17), all contain a single GU pair in their cloverleaf structures and all exhibit a resonance at about −10.5 ppm with intensity corresponding to approximately two protons. However yeast $tRNA^{Asp}$, which contains three GU pairs and 1 GΨ pair (18), contains four to five times more intensity in the −10 to −11 ppm region. Based on this correlation we tentatively conclude that GU pairs generate two proton resonances in the −10 to −11 ppm region. Additional support for this assignment has been obtained from the NMR melting experiments on yeast $tRNA^{Asp}$ in which these resonances melt synchronously with the base-paired proton resonances from the same helices (Robillard *et al.*, manuscript in preparation). It is clear from the above considerations that these are *not* ring NH protons hydrogen bonded to ring nitrogens as would be observed in either tautomeric form of the GU pairs. Alden and Arnott (19) have calculated the strains needed to accommodate a GU "wobble" pair in an RNA helix and conclude this fits well enough into an RNA helix so that very little backbone distortion would exist, as is observed in the crystal structure (5, 6). This would result in two similar bonds per GU pair between ring NH protons and exocyclic oxygens and could resemble the hydrogen bonds formed in the model system between the ring NH protons and the dimethyl sulfoxide solvent. Katz and Penman (20) showed that this system gave proton resonances in the vicinity of −10.5 ppm. Hence, the present results are not inconsistent with wobble GU pairs. In order to eliminate the possibility that the G and U residues are stacked but not hydrogen bonded at all, it would be necessary to have measurements of the ring NH positions in non-hydrogen bonding solvents under conditions which are presently not available.

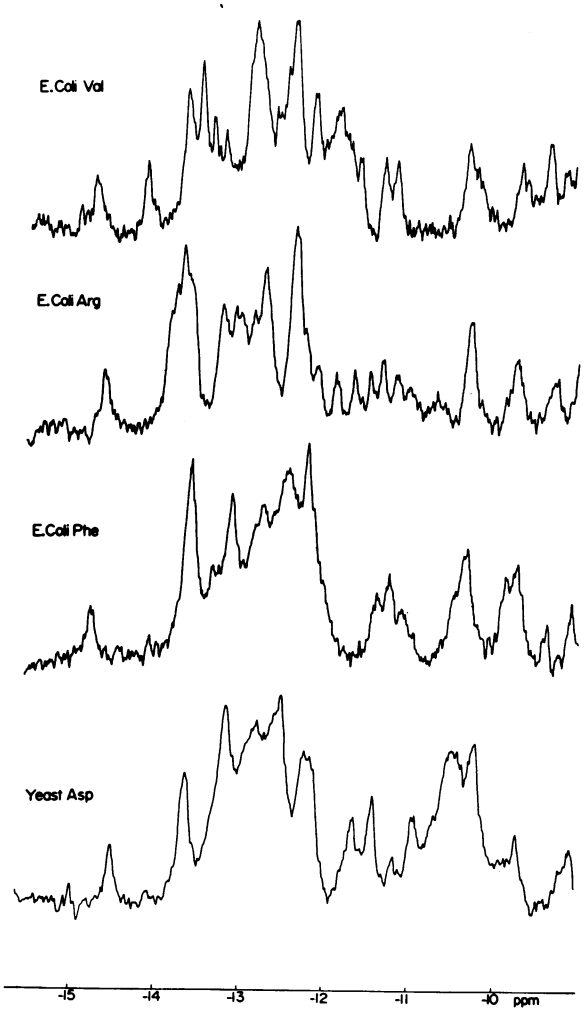

FIG. 2. 270 MHz proton NMR spectrum of three *E. coli* tRNAs and yeast $tRNA^{Asp}$ at 35°. The *E. coli* $tRNA^{Arg}$, although pure with respect to aminoacylation with arginine, is a mixture of at least two isoaccepting species. The yeast $tRNA^{Asp}$ was kindly provided by Dr. G. Dirheimer and will be the subject of a more detailed communication later.

Region between −11 and −15 ppm

Previous NMR spectra of several tRNAs and hairpin helices demonstrated that the ring NH hydrogen bond of A·U base pairs has an unshifted resonance position approximately 1 ppm to lower field than that of G·C pairs (3, 4). From these experiments it was deduced that the inherent resonance position of an A·U pair was −14.6 to −14.8 ppm and the actual observed resonance position was shifted at least 0.2 ppm upfield due to ring current shifts from neighboring bases. Thus A·U pairs were not observed lower than about −14.5 ppm in the yeast tRNAs we had studied. It has not been possible from this viewpoint to understand weak resonances which had previously been observed near −14.9 ppm in *E. coli* $tRNA^{fMet}$ (7, 21), *E. coli* $tRNA^{Arg}$ (7), and *E. coli* $tRNA^{Phe}$ (21). Because these resonances were of nonintegral intensity in the samples used previously their interpretation was difficult. In the present highly purified and functional samples of *E. coli* $tRNA^{Phe}$, *E. coli* $tRNA^{Arg}$, and *E. coli* $tRNA_1^{Val}$ the resonances observed near −14.9 ppm have intensities close to one full proton. Furthermore, the ring current calculations do not predict any resonance below −14.3 ppm in these three tRNAs. In fact only one resonance is expected in each of these three tRNAs below −13.9 ppm; thus a peak at −14.9 is very anomalous. The following observations led to a hypothesis concerning the origin of the low field resonance: (*i*) the −14.9 ppm resonance is not observed in yeast $tRNA^{Phe}$ (2, 3), yeast $tRNA^{Tyr}$ (5), yeast $tRNA^{Trp}$ (5), yeast $tRNA^{Asp}$ (see Fig. 2); the −14.9 ppm resonance is observed in *E. coli* $tRNA_1^{Val}$, *E. coli* $tRNA^{Phe}$, *E. coli* $tRNA^{Arg}$ (see Fig. 2), and a resonance is also observed at this position in *E. coli* $tRNA^{fMet}$ (7, 8). The latter *E. coli* tRNAs contain s^4U at position 8, whereas the former yeast tRNAs contain U at position 8. (*ii*) In dimethylsulfoxide solution the ring NH proton resonance of s^4U is about 1 ppm to lower field than the ring NH resonance of U. (*iii*) The crystal structure of yeast $tRNA^{Phe}$ contains a tertiary base pair between U at position 8 and A at position 14 (5, 6).

These three facts suggested that s^4U8 and A14 may be involved in a tertiary base pair in these three *E. coli* tRNAs and this base pair could generate a resonance below −14.5 ppm. In order to test this hypothesis experimentally we converted s^4U to U by the cyanogen bromide procedure of Walker and RajBhandary (12). Fig. 3A shows the near ultraviolet spectrum of *E. coli* $tRNA_1^{Val}$ before and after treatment with a slight molar excess of CNBr; it is apparent from the 335 nm absorbance that at least 85% of the s^4U has been converted to U and the tRNA is still functional after this treatment—see Fig. 3B.

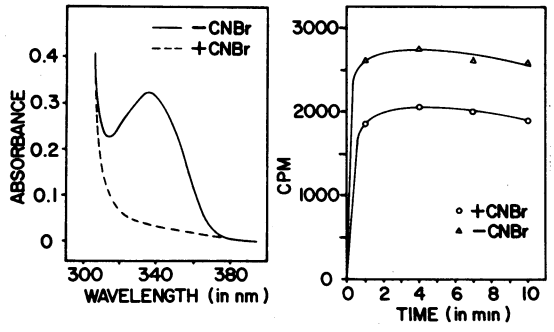

FIG. 3A (*left*). The near ultraviolet spectrum of *E. coli* $tRNA_1^{Val}$ before and after treatment with cyanogen bromide. Both samples were adjusted to an A_{260} of 15.0 (approximately 0.75 mg/ml). Fig. 3B (*right*). Aminoacylation of untreated and CNBr-treated *E. coli* $tRNA_1^{Val}$ with L-[^{14}C]valine by valyl-tRNA synthetase as described in *Materials and Methods*.

The NMR spectrum of dethiolated $tRNA_1^{Val}$ is shown in Fig. 4. The elimination of the −14.9 ppm resonance concomitant with sulfur removal in a tRNA *which is still functional* establishes that this resonance is in fact generated by a base pair involving s^4U at position 8. By analogy with the crystal structure of yeast $tRNA^{Phe}$ this interaction probably involves tertiary structure hydrogen bonding to A14. The resonances in the main region of the dethiolated spectrum are not as well resolved as in the control material, leaving the possibility that subtle changes in the overall conformation may result from sulfur removal; however, the most obvious change is the loss of the −14.9 ppm peak which we therefore assign to the thiouracil 8 to adenine 14 tertiary bond.

In our previous studies on other tRNAs we have focused our attention on the region between −11 ppm and −15 ppm (1–5); this region contains resonances from ring NH hydrogen bonds, provided their lifetime is longer than about 5 msec (8). Hence the number of protons in this region reflects the number of base pairs. Several of the smallest peaks in the spectrum of *E. coli* $tRNA^{Val}$ (Fig. 1) are of approximately equal intensity, e.g., peaks A, B, E, F, N, O, and P. On the assumption that each of these peaks corresponds to a single base pair, then the integrated intensity of the −11 to −15 ppm region indicates the presence of 26 base pairs, with an estimated error of about ±3 protons. Since the secondary structure of *E. coli* $tRNA_1^{Val}$ contains only 20 Watson–Crick base pairs, an extremely interesting conclusion to be drawn from this spectrum is that the solution structure of this tRNA contains several extra base pairs involving ring NH hydrogen bonds which could arise from tertiary structure; at least one, the 8 to 14 pair, has been experimentally shown to be derived from the tertiary structure.

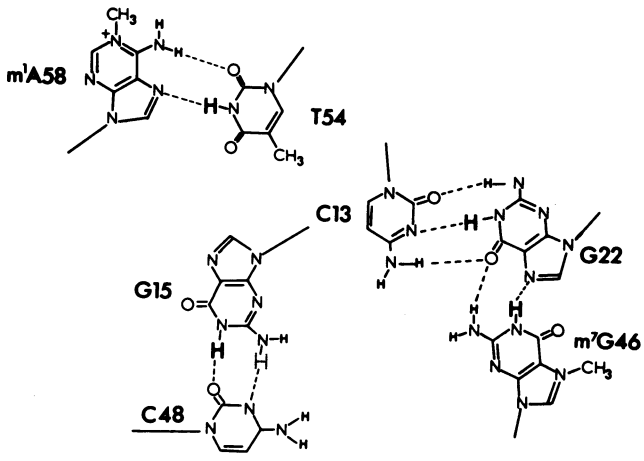

FIG. 5. Three of the tertiary interactions seen in the crystal structure of yeast $tRNA^{Phe}$. The hydrogen bonding data are taken from refs. 5 and 6. The hydrogens of ring NH-ring N bonds are shown in bold face; exocyclic NH_2-ring N bonds are dashed.

DISCUSSION

Our results on the proton NMR spectrum of *E. coli* $tRNA_1^{Val}$ demonstrate the presence of approximately four protons in the −9 to −10 ppm region which are at too high a field to be shifted ring NH protons. Thus we were forced to consider that they may be hydrogen bonded amino protons which are somehow strongly shifted downfield from their usual resonance position around −7.5 to −8 ppm (2). Katz and Penman observed that amino protons, when hydrogen bonded to exocyclic oxygen atoms, exhibited a downfield shift of only about 0.5 ppm; however the ring NH proton, when hydrogen bonded to a ring nitrogen, suffered a much larger downfield shift of about 1.5–2.5 ppm (20). Thus an amino proton hydrogen bonded to a ring nitrogen might be shifted into the −9 to −10 ppm region. Examination of the crystal structure of yeast $tRNA^{Phe}$ revealed the presence of such bonds; G15 forms a tertiary base pair with C48 but this is a reverse Watson-Crick pair involving trans glycosyl bonds. As shown in Fig. 5, the G15 exocyclic amino group hydrogen bonds to

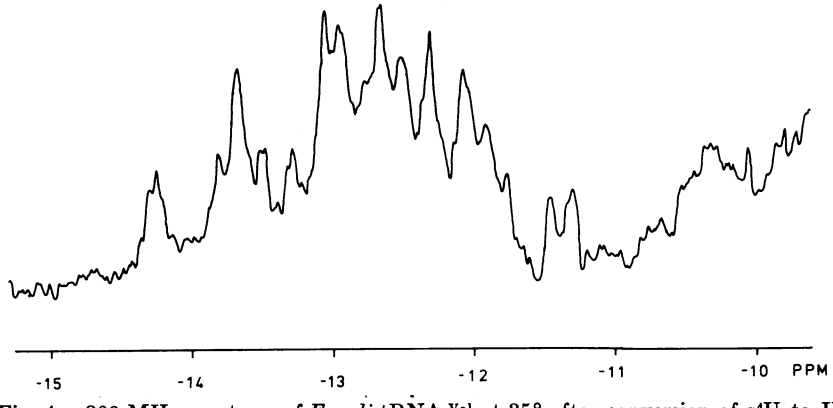

Fig. 4. 360 MHz spectrum of *E. coli* $tRNA_1^{Val}$ at 35° after conversion of s^4U to U.

the ring N3 of C48; conversely the ring NH of G15 is bonded to the exocyclic oxygen of C48 instead of to N3. Thus the G15 amino group might be downfield-shifted into the −9 to −10 ppm region. Similarly the bonding of A21 to the U8-A14 pair may involve a similar bond (5) and the bonding of A9 to the U12-A23 pair involves two such amino-ring N hydrogen bonds (5, 6). We feel these four tertiary hydrogen bonds are possible candidates for the four resonances in the −9 to −10 ppm region, since $tRNA_1^{Val}$ also contains G15 and C48, A21 and s^4U8-A14, and A9 and U12·A23.

Now that one resonance in the −11 to −15 ppm region has been experimentally assigned to the tertiary structure, and since the relative integrated intensities indicate more than 20 base pairs, we must ask what other candidates there are from the crystal structure for this spectral region. In addition to the s^4U8·A14, and G15·C48 bonds there are three more ring NH-ring N hydrogen bonds, the first of which is the Watson-Crick pair G19·C56. Another NH-ring N bond is derived from the ternary complex between m^7G46, G22, and C13. In this interaction the ring NH of m^7G46 is bonded to the N7 ring nitrogen of G22—see Fig. 5. A third candidate is the interaction T54–m^1A58 (A58 in *E. coli* $tRNA_1^{Val}$) in which the ring NH of T is bonded to a ring nitrogen; this is a Hoogsteen type pair involving N7 of A (5, 6)—see Fig. 5. In summary, the crystal structure reveals five candidates for tertiary resonances in this region.

In the past we have identified one G·C tertiary resonance in *E. coli* $tRNA^{Glu}$ and it is certainly possible that there is one additional G·C resonance in that spectrum. The possibility that there are extra resonances from tertiary base pairs in the −11 to −15 ppm region in all the tRNA spectra will be discussed in a more complete report.

With such good agreement between the NMR spectra and other measurements it is clear that in the future NMR can be even more useful in following structural features of tRNA in solution.

We thank Alex Rich and Aaron Klug for allowing one of us (B.R.R.) to examine in detail their models of the crystal structure of yeast $tRNA^{Phe}$. The support of the following grants (to B.R.R.) for the research carried out at Riverside is gratefully acknowledged: U.S. Public Health Service Grant CA-11697-04 and National Science Foundation Grant GB-41585.

1. Kearns, D. R., Patel, D. & Shulman, R. G. (1971) *Nature* **229**, 338–339.
2. Wong, Y. P., Kearns, D. R., Reid, B. R. & Shulman, R. G. (1972) *J. Mol. Biol.* **72**, 725–740.
3. Lightfoot, D. R., Wong, K. L., Kearns, D. R., Reid, B. R. & Shulman, R. G. (1973) *J. Mol. Biol.* **78**, 71–89.
4. Hilbers, C. W. & Shulman, R. G. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3239–3242.
5. Kim, S. H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wang, A. H. J., Seeman, N. C. & Rich, A. (1974) *Science* **185**, 435–440.
6. Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C. & Klug, A. (1974) *Nature* **250**, 546–551.
7. Shulman, R. G., Hilbers, C. W., Kearns, D. R., Reid, B. R. & Wong, Y. P. (1973) *J. Mol. Biol.* **78**, 57–69.
8. Crothers, D. M., Cole, P., Hilbers, C. W. & Shulman, R. G. (1974) *J. Mol. Biol.* **87**, 63–88.
9. Wimmer, E., Maxwell, I. H. & Tener, G. M. (1968) *Biochemistry* **7**, 2623–2628.
10. Nishimura, S. (1971) in *Procedures in Nucleic Acid Research*, eds., Cantoni, G. L. & Davies, D. R. (Harper & Row, New York), Vol. 2, pp. 542–564.
11. Pearson, R. L., Weiss, J. F. & Kelmers, A. D. (1971) *Biochim. Biophys. Acta* **228**, 770–774.
12. Walker, R. T. & RajBhandary, U. L. (1972) *J. Biol. Chem.* **247**, 4879–4892.
13. Reid, B. R. (1968) *Biochem. Biophys. Res. Commun.* **33**, 627–635.
14. Yaniv, M. & Barrell, B. G. (1969) *Nature* **222**, 278.
15. Kimura, F., Harada, F. & Nishimura, S. (1971) *Biochemistry* **10**, 3277–3283.
16. Barrell, B. G. & Sanger, F. (1969) *FEBS Lett.* **3**, 275–278.
17. Murao, K., Tanabe, T., Ishii, F., Namiki, M. & Nishimura, S. (1972) *Biochem. Biophys. Res. Commun.* **47**, 1132–1137.
18. Gangloff, J., Keith, G., Ebel, J. P. & Dirheimer, G. (1972) *Biochim. Biophys. Acta* **259**, 198–209.
19. Alden, C. J. & Arnott, S. (1973) *Biochem. Biophys. Res. Commun.* **53**, 806–811.
20. Katz, L. & Penman, S. (1966) *J. Mol. Biol.* **15**, 220-231.
21. Kearns, D. R., Patel, D., Shulman, R. G. & Yamane, T. (1971) *J. Mol. Biol.* **61**, 265-270.